Tissue Cultures of Phaseolus Review and Recent Developments

Machteld C. Mok and David W. S. Mok

Department of Horticulture and Genetics Program Faculty, Oregon State University, Corvallis, Oregon 97331

I. Brief Review and Current Status of:

A. Callus Cultures: Extensive efforts to culture callus tissues of Phaseolus vulgaris were carried out by Liau and Boll (10). The inorganic constituents of Murashige and Skoog's medium (14) were found to be more suitable than those contained in White's medium (16) in supporting callus growth. Suspension cultures were sustained with a 1/4 strenght MS medium (10) or a modified B_5 medium (5, 11). However, for rapid proliferation of callus, series of undefined and defined organic compounds, such as coconut milk, amino acids and vitamins were necessary. The minimal organic requirements of the medium were re-examined in our laboratory and the vitamins thiamine, nicotinic acid and pyridoxine were found to be essential. The types of auxin and cytokinin and the respective concentrations required to support callus proliferation were dependent on the genotype of the plant material (12, 13). In addition, the auxin picloram stimulated growth of Phaseolus tissues, especially those of P. lunatus, over a wide range of concentrations (12).

B. Redifferentiation: Regeneration of plants from callus tissue of Phaseolus, as in many other leguminous species, has not been successful. It appears that the usual manipulations of cytokinin and auxin concentrations as practiced in other plant species, is not sufficient for redifferentiating Phaseolus tissues. However, organization of "nodules" containing xylem and phloem in P. vulgaris has been reported (6, 8). We have observed the formation of "embryo-like" structures in several Phaseolus species. Root formation can be easily induced in most genotypes and is not a limiting factor in the regeneration of plants. Shoot formation was documented with the addition of bean seed extract (4), and shoots were obtained on defined medium in our laboratory. However, the results are not consistant and further investigation is warranted.

C. Protoplast Manipulation: Protoplasts can be derived from leaf mesophyll cells of Phaseolus with the procedures established for higher plants (15). Protoplast fusion with polyethylene glycol (PEG) between genera has been performed (3, 9). Successful somatic hybridization with a selection technique as demonstrated in Nicotiana (1) has not been reported for Phaseolus. The value of future application of protoplast manipulation would be dependent on the ability to regenerate plants from callus tissues.

D. Embryo Culture: The utilization of embryo cultures in Phaseolus for the development of interspecific hybrids was first reported by Honma (7). White's medium supplemented with coconut milk, nicotinic acid and thiamine was used. Recently, systematic efforts were directed at the investigation of developmental events in P. coccineus using embryo culture (2). It was noted that embryos before or at heart-shape stage required the association of suspensors for normal development in culture, while more mature embryos did not have such requirements. The addition of gibberellin to the culture medium replaced the need of young embryos for suspensors. Glutamine and nicotinic acid appeared to be beneficial as well. Results obtained in our laboratory suggest that embryos of the species P. vulgaris, P. lunatus and P. acutifolius develop normally on MS medium supplemented with vitamins (nicotinic acid, thiamine and pyridoxine) and glutamine. The effect of gibberellin on small embryos was not apparent, and no species-specific requirements were found. Plantlets have been established from hybrid embryos derived from matings between P. vulgaris x P. lunatus and P. vulgaris x P. acutifolius. Based on these findings, the composition of culture medium does not appear to be the critical factor for success. The identification of parental combinations producing high frequency of hybrid embryos and the improvement of survival of seedlings during transition period (from sterile to normal environment) would permit the routine generation of interspecific hybrids.

II. Essential Considerations:

(1) Intra- and inter-specific comparisons suggest that the differential auxin requirements between Phaseolus genotypes can be as great as 20-fold. Although the majority of genotypes within the same species may have similar auxin requirements, the responses are not species specific. As the auxin picloram is effective over a wide range of concentrations, a particular concentration of picloram may be suitable for more genotypes than a concentration of 2, 4-D. These observations coupled with the established differences in cytokinin structure-activity relationships between species (see section III) , suggest that the genotypic properties of the experimental materials should be of primary concern.

(2) The origin of the tissue (hypocotyl, cotelydon, etc.) has slight influence on the growth regulator concentrations required to initiate cultures. Callus tissues at subsequent passages appear to have identical hormonal requirements regardless of their source of origin.

(3) The duration of the culturing period, or the number of passages, may greatly influence phenotypic traits such as autotrophism, and the ability to form roots and shoot primordia. These phenotypic expressions in subcultures are dependent on the genotype.

III. Phaseolus Tissue Culture as a Bioassay System:

The manipulation of Phaseolus tissues in culture provides a useful tool in the investigation of the genetic regulation of hormonal function and metabolism. Genotypes with extreme differences in the concentration of auxin required for optimal growth have been identified, and the inheritance of such differences is being investigated. Genotypic variations in responses to cytokinins, as determined by cytokinin structure-activity relationships, were discovered. The activities of cytokinins with a saturated N^6 - isoprenoid side chain (such as dihydrozeatin and N^6 - isopentyladenine) are 30 to 100 fold more active in genotypes of P. vulgaris than cytokinins with an unsaturated N^6 side chain (such as zeatin and N^6-(Δ^2 - isopentenyl) adenine), whereas the order of relative activities was reversed in genotypes of P. lunatus. The ability to identify and define genotypic variations in hormonal responses using tissue culture technique would render Phaseolus a valuable system in relating genetic regulation to physiological expression.

References

1. Carlson, P. S., H. H. Smith and R. D. Dearing. 1972. Parasexual interspecific plant hybridization. Proc. Nat. Acad. Sci. (USA) 69:2292-2294.

2. Cionini, P.G., A. Bennici, A. Alpi and F. D'Amato. 1976. Suspensor, gibberellin and in vitro development of Phaseolus coccineus embryos. Planta 131:115-117.

3. Constabel, F. and K. N. Kao. 1974. Agglutination and fusion of plant protoplasts by polyethylene glycol. Can. J. Bot. 52:1603-1606.

4. Crocomo, O. J., W. R. Sharp and J. E. Peters. 1976. Plantlet morphognesis and the control of callus growth and root induction of Phaseolus vulgaris with the addition of a bean seed extract. Z. Pflanzenphysiol. 78: 456-460.

5. Gamborg, O. L., R. A. Miller and K. Ojima. 1968. Nutrient requirements of suspension culture of soybean root cells. Exptl. Cell Res. 50:151-158.

6. Haddon, L. and D. H. Northcote. 1976. The effect of growth conditions and origin of tissue on the ploidy and morphogenetic potential of tissue cultures of bean (Phaseolus vulgaris L.). J. Exp. Bot. 27:1031-1051.

7. Honma, S. 1956. A technique for artificial culturing of bean embryos. Proc. Amer. Soc. Hort. Sci. 65: 405-408.

8. Jeffs, R. A. and D. H. Northcote. 1967. The influence of indole-3-yl-acetic acid and sugar on the pattern of induced differentiation in plant tissue culture. J. Cell Sci. 2: 77-88.

9. Kao, K. N., F. Constabel, M. R. Michayluk, and O. L. Gamborg. 1974. Plant protoplast fusion and growth of intergeneric hybrid cells. Planta 120: 215-227.

10. Liau, D. F. and W. G. Boll. 1970. Callus and cell suspension cultures of bush bean (Phaseolus vulgaris). Can. J. Bot. 48:1119-1130.

11. Mante, S. and W. G. Boll. 1975. Comparison of growth and extracellular polysaccharide of cotyledon cell suspension cultures of bush bean (Phaseolus vulgaris cv. Contender) grown in coconut milk medium and synthetic medium. Can. J. Bot. 53:1542-1548.

12. Mok, M. C. and D. W. S. Mok. 1977. Genotypic responses of Phaseolus tissues to auxin. Physiol. Plant. 40: 261-264.

13. Mok, M. C., D. W. S. Mok and D. J. Armstrong. 1978. Differential cytokinin structure-activity relationship in Phaseolus. Plant Physiol. (in press).

14. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

15. Pelcher, L. E., O. L. Gamborg, and K. N. Kao. 1974. Bean mesophyll protoplasts: production, culture, and callus formation. Plant Sci. Letters 3: 107-111.

16. White, P. R. 1943. Handbook of plant tissue culture. G. Cattell, Lancaster, Pa.

* * * * * * * * * * * *